REMARKS

Claims 1-18 are pending and have been finally rejected. Claims 20-51 have been withdrawn subject to an earlier restriction requirement. No claims have been allowed.

Claim 1 has been amended to make it clearer that sulfonhydrazone is reacted with a sulfonyl halide. Support is found throughout the specification and claims as originally filed, fore example, claim 1 and Example 8. Applicants reserve the right to file the original scope of the claims in one or more continuing applications. No new matter is entered upon entry of these amendments.

A Request for Continued Examination (RCE) accompanies this amendment and response.

Applicants thank Examiners Rebecca Fritchman and Krishnan Menon for the telephonic interview with Attorney Jeffrey H. Rosedale. It was discussed that applicants would be filing this amendment and RCE so that the examiners may further consider the claims, as presently amended, in view of the prior art of record.

I. Rejections under 35 U.S.C. § 103

Claims 1-9, 12-14 and 16-18 stand finally rejected as allegedly being obvious over Visser et al. in view of Bailey et al., and in further view of Takadate et al., the citations of which documents are already of record. Claims 1-18 also stand finally rejected as allegedly being obvious over Berliner in view of Bailey, and in further view of Takadate, the citations of which documents are already of record.

Applicants traverse the rejection on the grounds that the alleged combination of Visser and Bailey fails to provide all of the elements of Applicants' invention as claimed. For example, claim 1, as presently amended, requires that the sample is reacted with a sulfonyl halide following reacting the sample with the sulfonhydrazide. Because neither Visser nor Bailey discloses reacting a sample with a sulfonyl halide following reacting the sample with the sulfonhydrazide, Applicants submit that the rejection should be withdrawn.

Applicants traverse the rejection on the grounds that the invention is not obvious as the cited prior art does not teach or suggest to one of ordinary skill in the art to react a

ketosteroid with a sulfonhydrazide to form a sulfonhydrazone **and subsequently** to react the resulting sulfonhydrazone with a sulfonyl halide. The Office Action at page 5, lines 4-10 states that

> "Takadate et al. teach that derivatization of ketosteroid estrogens using a sulfonyl halide is an alternative derivatization to that of ketosteroids using sulfonhydrazides. It would have been obvious to one of ordinary skill in the analytical chemistry arts ***to follow*** the derivatization of ketosteroids using sulfonhydrazides with an ***alternative*** and well-known method of derivatization of ketosteroids using sulfonyl halide. ***The purpose or advantage of doing so would be to check the relative accuracy of the first method.***" (emphasis supplied)

Applicants respectfully submit that this reasoning is incorrect. In fact, the purpose or advantage of reacting a ketosteroid with a sulfonhydrazide to form a sulfonhydrazone and subsequently reacting the resulting sulfonhydrazone with a sulfonyl halide is quite different than that stated in the Office Action. As well, Applicants have observed surprising results in carrying out their method. According to the specification at paragraph **[0138]**, with combined sulfonhydrazide derivatization of the carbonyl and sulfonyl derivatization of the hydroxyl (e.g., as exemplified in Applicants' Example 8 with TSH and dansyl chloride, respectively), all estrogens and estrogen metabolites show intense protonated molecule [MH^+] and less abundant natriated molecule [MNa^+] during ESI positive ion mode. In contrast, some D-ring ketolic estrogen metabolites, such as 1 6α-hydroxyestrone, 16-ketoestradiol, 2-hydroxyestrone, and 4-hydroxyestrone, show rather poor response and hydrogen loss when derivatized with the dansyl chloride procedure alone without prior carbonyl protection **(FIG. 8)**, which is believed to be due to their alkali labile nature. The extensive hydrogen loss found in deuterium-labelled D-ring ketolic estrogen metabolites with dansyl chloride derivatization alone is especially problematic since ion clusters from their protonated or natriated molecules also cover those of target analytes (**FIGS. 8 and 9**), which makes accurate quantitative measurement difficult.

Unlike the dansyl chloride derivatization alone, the combined TSH carbonyl protection and dansyl chloride derivatization is a method suitable for the quantitative measurement of all endogenous steroids (such as estrogens and estrogen metabolites) by HPLC-ESI-MS. (Specification, paragraph **[0138]**).

Hence, Applicants' claimed two-step process of carbonyl derivatization followed by hydroxyl derivatization provides for better HPLC separation of steroids, and allows for better signal detection in API-MS (such as ESI-MS) when at least one of the derivatization groups contains a highly ionizable moiety that enables ionization under either positive ion or negative ion mode of electrospray ionization. Because neither Visser in view of Bailey, and further in view of Takadate, nor Berliner in view of Bailey, and further in view of Takadate discloses reacting a sample with a sulfonyl halide followed by reacting the sample with the sulfonhydrazide ***in order to improve signal detection in API-MS***, Applicants submit that the rejection should be withdrawn.

The Commissioner is hereby authorized to charge any fee deficiency, charge any additional fees, or credit any overpayment of fees, associated with this application in connection with this filing, or any future filing, submitted to the U.S. Patent and Trademark Office during the pendency of this application, to Deposit Account No. 23-3050.

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